

Amendment to the Claims

1. (Currently Amended) A process for isolating an intact clone of one target nucleic acid fragment having a known characteristic, from a number of fragments capable of containing said target nucleic acid fragment, said process comprising:

- a) identifying a target nucleic acid fragment having a known characteristic;
- b) providing a number of nucleic acid fragments,
- c) preparing an initial library of clones from said number of fragments using a vector containing no more than a pre-determined number of known restriction sites;
- d) subjecting said initial library to a plurality of restriction enzymes individually, which plurality of enzymes do not include those to which said vector is sensitive, to produce a group of monodigested libraries;
- e) screening said group of monodigested libraries for said known characteristic to detect the presence of intact target fragments, to thereby determine those restriction enzymes to which said target fragment is insensitive;
- f) subjecting said initial library to substantially all of said plurality of restriction enzymes to which said target fragment is insensitive, to produce a multidigested library having an intact clone of the target nucleic acid fragment; and
- g) isolating an intact clone from the multidigested library.

2. (Previously amended) The process of Claim 1 wherein said plurality of restriction enzymes comprises at least 10 restriction enzymes.

3. (Previously amended) The process of Claim 1 wherein said plurality of restriction enzymes comprises at least 50 restriction enzymes.
4. (Previously amended) The process of Claim 1 wherein said plurality of restriction enzymes comprises at least 70 restriction enzymes.
5. (Previously amended) The process of Claim 1 wherein said pre-determined number of known restriction sites is four.
6. (Previously amended) The process of Claim 1 wherein said pre-determined number of known restriction sites is three.
7. (Previously amended) The process of Claim 6 wherein at least one of said three sites is different from, and flanked by, said two remaining sites.
8. (Original) The process of claim 1 wherein said restriction enzymes have cleavage sites from 5 to 6 nucleotides in length.
9. (Previously amended) The process of Claim 1 including the further step of transforming and replicating said intact clone of the target nucleic acid fragment.
10. (Previously amended) The process of Claim 9 including the further step of isolating said intact clone.
11. (canceled).
12. (Previously amended) The process of Claim 1 comprising, after step b), the further step of transfecting said monodigested libraries in cellular hosts.
13. (Previously amended) The process of Claim 1 comprising the further step of verifying the presence of said target fragment in said initial library by transfecting in a cellular host and screening said transfected host for the presence of said target fragment.

14. (Previously amended) The process of Claim 1 comprising the further step of verifying the presence of said target fragment in said multi-digested library by transforming said library and screening said transformed library for the presence of said target fragment.

15. (Previously amended) The process of Claim 1 wherein said number of fragments contains up to 10^8 fragments, each from about 0.1kb to 5kb in size.

16. (Currently amended) A process for isolating an intact clone of one target nucleic acid fragment capable of containing said target nucleic acid fragment having a known characteristic, from a group of fragments, said process comprising:

- a) identifying a target nucleic acid fragment having a known characteristic of interest;
- b) providing a number of said nucleic acid fragments;
- c) preparing an initial library of clones from said number of fragments using a vector containing no more than a pre-determined number of known restriction sites;
- d) verifying the presence of said target fragment in said initial library by transfecting in a cellular host and screening said transfected host for the presence of said target fragment;
- e) subjecting said initial library to a plurality of restriction enzymes individually, which plurality of enzymes do not include those to which said vector is sensitive, to produce a group of monodigested libraries;
- f) independently transfecting said monodigested libraries;
- g) screening said group of monodigested libraries for said known characteristic to detect the presence of intact target fragments, to thereby determine those restriction enzymes to which said target fragment is insensitive;

h) subjecting said initial library to substantially all of said plurality of restriction enzymes to which said target fragment is insensitive, to produce a multidigested library having an intact clone of the target nucleic acid fragment; and

i) transforming said multidigested library.

17. (Previously amended) The process of Claim 16 wherein said restriction enzymes have cleavage sites from 5 nucleotides in length.

18. (Original) A process for isolating an intact clone of one target nucleic acid fragment having a known characteristic, from a group of fragments, said method comprising:

a) preparing an initial library of clones from said group of fragments using a vector containing no more than a predetermined number of known restriction sites;

b) subjecting said initial library to a plurality of restriction enzymes individually, which plurality of enzymes do not include those to which said vector is sensitive, to produce a group of mono-digested libraries;

c) transforming said monodigested libraries into bacteria;

d) culturing said bacteria to produce digested libraries substantially free of cleaved products, cleaving each digested library to produce digestion products, depositing said products in an agarose gel well, migrating said products, transferring said products onto a membrane, hybridizing said transferred products with a probe, to thereby determine those restriction enzymes to which said target fragment is insensitive; and

d) subjecting said initial library to substantially all of said plurality of restriction enzymes to which said target fragment is insensitive, to produce a multi-digested library having an intact clone of the target nucleic acid fragment.

19. (Original) A method for producing a series of monodigested libraries from a group of fragments, said method comprising:

- a) preparing and initial library of clones from said group of fragments using a vector containing no more than a pre-determined number of known restriction sites; and
- b) subjecting said initial library to a plurality of restriction enzymes individually, which plurality of enzymes do not include those to which said vector is sensitive, to produce a group of monodigested libraries.

20. (Withdrawn from Consideration)

21. (Cancelled).

22. (Cancelled).

23. (New) A process for efficiently constituting expression libraries and isolating a target gene of interest, said process comprising:

- a) identifying a cDNA of a tissue of interest or cell line of interest with a target activity or phenotype;
- b) providing a vector,
- c) preparing a library by inserting said cDNA in site A of the vector;
- d) verifying presence of said cDNA by transfecting in a cell line lacking said target activity or phenotype and measuring restoration of the target activity or phenotype;
- e) digesting said library with each of all known restriction enzymes resulting in a plurality of monodigested libraries;

f) transfected each said monodigested library into independent cell lines;

g) testing for the presence of said target activity or phenotype in each of said cell lines;

h) establishing a multiple enzymatic characteristic of the target activity or phenotype by recording the sensitivity to each restrictive enzyme;

i) digesting the library with all restriction enzymes to which the target activity or phenotype is resistant, thereby obtaining multidigested libraries;

j) transforming said multidigested libraries in competent bacteria cells;

k) subcloning using enzyme B in a vector; and

l) sequencing do not include those to which said vector is sensitive, to produce a group of monodigested libraries;

m) screening said target gene of interest.